

### Anti-PEG IgE in anaphylaxis associated with polyethylene glycol



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#### Clinical Implications

- The dual cytometric beads assay described in this report demonstrates that IgE type 1 hypersensitivity is a mechanism of polyethylene glycol-associated anaphylaxis. This new detection method could be a useful tool both for clinical practice and in screening for at-risk subjects.

Polyethylene glycol (PEG) has been used in a wide range of medical and pharmaceutical products as an active ingredient or excipient. In addition, an increasing number of PEG-modified (PEGylated) therapeutic proteins and drugs are being developed and approved for marketing.<sup>1</sup> The covalent attachment of PEG to a drug or therapeutic protein increases hydrodynamic size and can increase half-life.

Despite the benefit, PEG and PEGylated products are not free of risk and immune-mediated adverse events are of concern. There are an increasing number of PEG-associated anaphylaxis case reports,<sup>2</sup> many of which demonstrate clinical cross-reactivity with polysorbates.<sup>3</sup> Serious allergic reactions have been seen in patients who received PEG-L-asparaginase,<sup>4</sup> PEGylated IFN- $\alpha$ ,<sup>5</sup> and PEGylated G-CSF (pegfilgrastim), pegvaliase-pqzp, and peginesatide.<sup>6</sup> In some cases of PEG-associated reactions, an immediate skin test response suggests IgE-mediated type 1 hypersensitivity reactions. However, a reliable specific assay to sensitively detect specific preexisting anti-PEG IgE was not available to evaluate these events.

Most reported adverse events occurred on an apparent first exposure to a parenteral version of a specific-PEG-containing product,<sup>6</sup> suggesting previous sensitization to PEG. Data on sensitization to PEG in samples reflective of a broader population would be of value.

We developed a Dual Cytometric Bead Assay (DCBA) for anti-PEG IgG, IgM, and IgE in patient sera. Target beads and control beads were generated, incubated with samples, and washed as described in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org). Antihuman IgE-phycoerythrin (PE), antihuman IgGFc-PE, or antihuman-IgM-V450 were added and analyzed by flow cytometry after washing. Single-bead populations were gated by forward scatter and side scatter. Target beads and control beads were separated by allophycocyanin fluorescence intensity. PE fluorescence was compared between target and control beads. Flow cytometry data were analyzed with FlowJo software (FlowJo, LLC, Ashland, OR); more than 1000 signal events were collected per sample. Plasma or serum samples

from cases and controls were evaluated with pegloticase beads, and normal individuals were evaluated with peginesatide beads. Positive sera were serially diluted to determine antibody titers, and specificity was confirmed by competition with free PEG.

We used the DCBA to test anti-PEG antibodies in anaphylactic patients and controls. The exposures of anaphylaxis patients and controls are described in [Table E1](#) in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org). Details of clinical symptoms and skin tests of some of the anaphylaxis patients have been published in case reports.<sup>2,7</sup> Plasma or serum samples from cases and controls were collected at various time points after the last episode or exposure, blinded, and sent to the Food and Drug Administration laboratory for anti-PEG IgE testing. In addition to the clinical samples, serum or plasma samples from approximately 2000 individuals with or without known disease background were purchased from BioIVT (Westbury, NY) and Equitech Enterprises, Inc (Kerrville, Texas). There was no information on previous exposure to PEG or allergic reaction to PEG.

Biospecimens were collected under an institutional review board-approved protocol and/or with patient consent as indicated in previously published case reports. Deidentified case and control samples from Vanderbilt University were collected under Vanderbilt University #150754, and #131836. The Food and Drug Administration laboratory evaluated remnant deidentified biospecimens and the Food and Drug Administration Institutional Review Board made a "not human subject research" determination.

To determine whether PEG and PEGylated drug-associated anaphylaxis is due to specific IgE-mediated type 1 hypersensitivity, we tested serum samples from patients with documented PEG-associated anaphylaxis for specific anti-PEG IgE. We obtained 9 patient samples from 2 clinical units. The samples included cases of PEG-associated anaphylaxis and controls from PEG-exposed patients without associated allergic symptoms.<sup>2,7,8</sup> The sources of PEG exposure included a visualization agent for echocardiograms, PEG 3350 in colonoscopy preparations and as an excipient, and a PEG 8000 lubricating gel. The samples for cases and controls were blinded for testing using the DCBA.

As summarized in [Table I](#), all the anaphylaxis case samples and none of the control samples were clearly positive for anti-PEG IgE. Samples from anaphylaxis cases also had high titers of anti-PEG IgG. Although subjects positive for anti-PEG IgE also had high anti-PEG IgG titers, the reverse was not true. Except for 1 case (case PEG9) with an anti-PEG IgM titer of less than 10, all other cases were anti-PEG IgM negative.

An example titration for a positive sample (PEG1) is shown in [Figure 1](#). The bell-shaped binding curve indicates inhibition of binding at very low dilutions. A control sample PEG3 had a marginal anti-PEG IgE signal with a difference between target beads and control beads well below assay variability (see [Figure E1](#) in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)). The PEG1 positive signal was fully inhibited by free PEG (50  $\mu$ g/mL), demonstrating specificity.

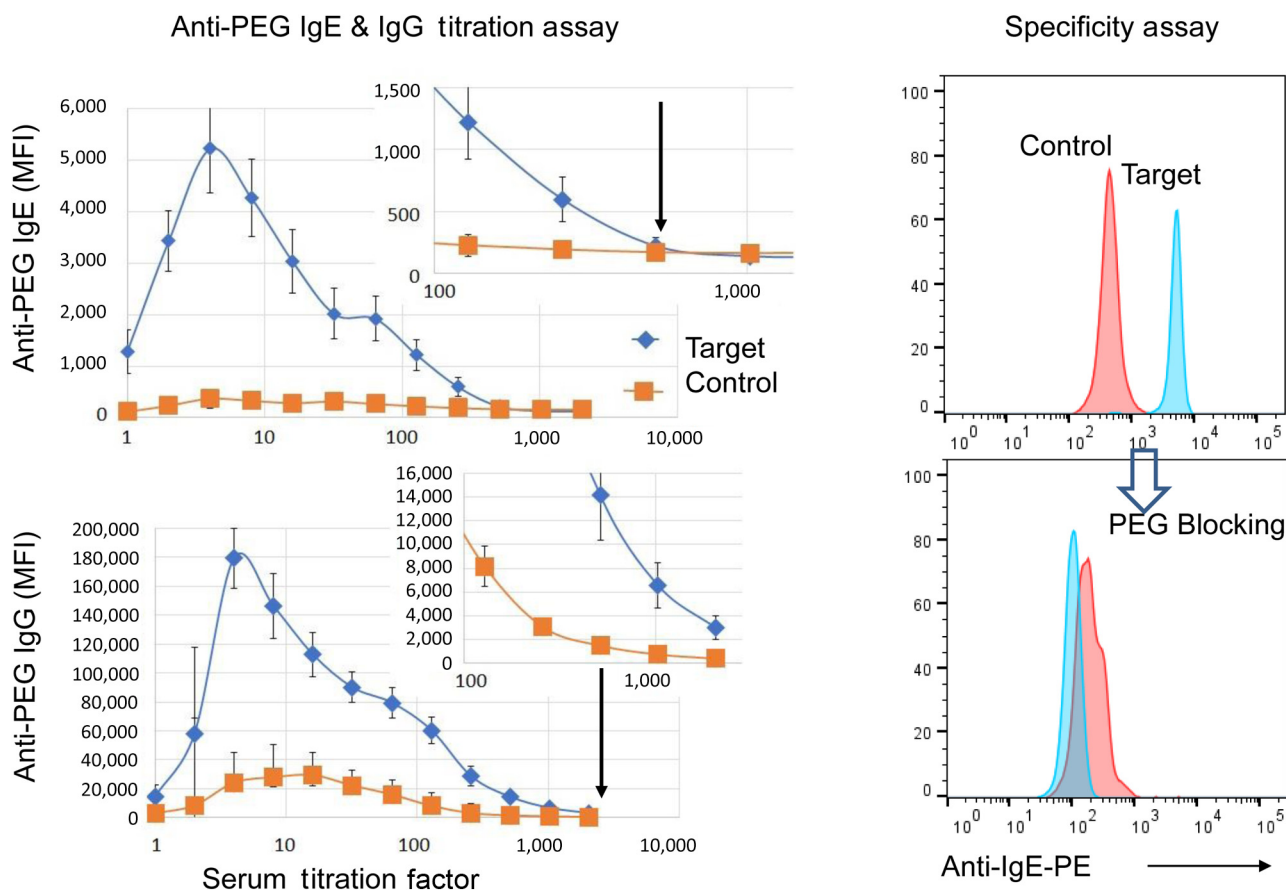
PEG-associated anaphylaxis cases often occurred on a "first exposure" to PEG or PEGylated drugs. To look for preexisting anti-PEG antibodies, we screened normal serum samples for

**TABLE I.** Anti-PEG IgE and anti-PEG IgG in sera from cases and controls of PEG-associated anaphylaxis

Lab ID	Clinic	Anti-PEG IgE			Anti-PEG IgG		
		Positivity (Max MFI)	Titration	Inhibition	Positivity (Max MFI)	Titration	Inhibition
PEG1	Case	+++ (4,855)	>512	100%	++ (154,969)	>16,384	100%
PEG2	Case	+ (1,076)	>32	100%	++ (109,079)	>8,192	100%
PEG3	Control	+/- (295)	>4	ND	+ (39,826)	>2,048	100%
PEG4	Control	- (-86)		ND	- (130)	1	ND
PEG5	Control	- (0)		ND	- (4,603)	>1	ND
PEG6	Case	++ (493)	>90	100%	+++ (40,419)	>10,000	100%
PEG7	Case	++ (291)	>100	100%	+++ (78,647)	>10,000	100%
PEG8	Case	++ (1,800)	>90	100%	+ (29,494)	>2,500	100%
PEG9	Case	+ (4,058)	>30	100%	++ (160,690)	>6,000	100%

MFI, Median fluorescence intensity; ND, not done.

Note: Number of “+” was assigned on the basis of titer; for IgE, a titer >30 is +, >90 is ++, and >512 is +++; for IgG, a titer >2,000 is +, >6,000 is ++, and >10,000 is +++.



**FIGURE 1.** Detection of anti-PEG IgE and IgG antibodies in patients who experienced anaphylaxis to PEG. Anaphylaxis case sample, PEG1, is positive for both anti-PEG IgE and IgG, with bell-shaped titration curves. Histograms of anti-PEG IgE signals for PEG1 are shown (blue for target beads and red for control beads). The signal was inhibited by free PEG. MFI, Median fluorescence intensity.

anti-PEG IgG, IgM, and IgE. We found that 5% to 9% of 1721 tested serum samples were positive for anti-PEG IgG and 3% to 6% of 948 were positive for anti-PEG IgM. This range is based on 2 screening thresholds (ie, 60% and 100% signals increase of target beads from control beads). We also found 2 of 2091

samples positive for anti-PEG IgE. All the antibodies tested were specific for PEG, as demonstrated by free PEG inhibition.

A review of the literature for anti-PEG antibody detection notes that most, if not all, assays, including commercial kits, are flawed and lack specificity.<sup>9</sup> This was addressed by the use of a

DCBA for the detection of anti-PEG antibodies, including anti-PEG IgE. Samples for PEG-associated anaphylaxis cases were positive for anti-PEG IgE, confirming the long-considered hypothesis of IgE-mediated type 1 hypersensitivity. We also demonstrated preexisting antibodies to PEG in normal sera, a potential explanation for reactivity on first known exposure. Preexisting anti-PEG in normal sera suggest a broad exposure to PEG in daily life. DCBA methods may be of significant value in verifying the diagnosis of suspected IgE-mediated PEG hypersensitivity and in identifying previously sensitized patients at risk for anaphylaxis with PEG products.

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Conflicts of interest: G. Sussman declares the following interests: advisory board member for Novartis, Aralez, CSL Behring, and Sanofi; received grant or honorarium from Novartis, Aralez, Pediapharm, GlaxoSmithKline (GSK), Genentech, DBV Technologies, Aimmune, CSL Behring, AstraZeneca, Stallergenes, Merck,

Pfizer, Dyax, BioCryst, Greencross, Kendrion, Shire, Leopharma, Regeneron, and mdBriefCase; and currently participating or have participated in clinical trial (principal investigator) for Novartis, GSK, Genentech, DBV Technologies, Aimmune, CSL Behring, Astrazeneca, Stallergenes, Merck, Pfizer, Dyax, Biocryst, Greencross, Kendrion, Leo Pharma, Regeneron, Sanofi, Blueprint, ALK, Amgen, and Cliantha. E. J. Phillips has received royalties from Uptodate, consultancy fees from Biocyst and Janssen, and has a patent for HLA-A\*32:01 for vancomycin hypersensitivity testing and is codirector of IIID Pty ltd, which has a patent for HLA-B\*57:01 testing for abacavir hypersensitivity. The rest of the authors declare that they have no relevant conflicts of interest.

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## ONLINE REPOSITORY

### METHODS

Functional cytometric bead array (CBA) beads E4 and E8 were purchased from BD Biosciences (San Jose, Calif). Mouse anti-PEG IgG mAbs were purchased from Life Diagnostics, Inc (West Chester, Pa). Biotin-conjugated anti-PEG mAb was obtained from LifeSpan BioSciences, Inc (Seattle, Wash). Streptavidin-PE, antihuman IgG-PE, antihuman IgM-AF450, antihuman-IgE-PE, biotinylated antihuman IgG, and biotinylated antihuman IgE mAbs were purchased from BD Biosciences or BioLegend (San Diego, Calif). The specificity of these reagents was validated by flow cytometry. Peginesatide (Omonlys) was obtained directly from the manufacturers (Affymax, Inc, & Takeda Pharmaceutical Company Ltd, San Diego, Calif), distribution sites, or from the field by Food and Drug Administration investigators. Other PEGylated drugs including peginterferon alfa-2b (PegIntron), pegfilgrastim (Neulasta), peginterferon alfa-2a (Pegasys), and pegloticase (Krystexxa) were obtained through the National Institutes of Health Pharmacy (Bethesda, Md).

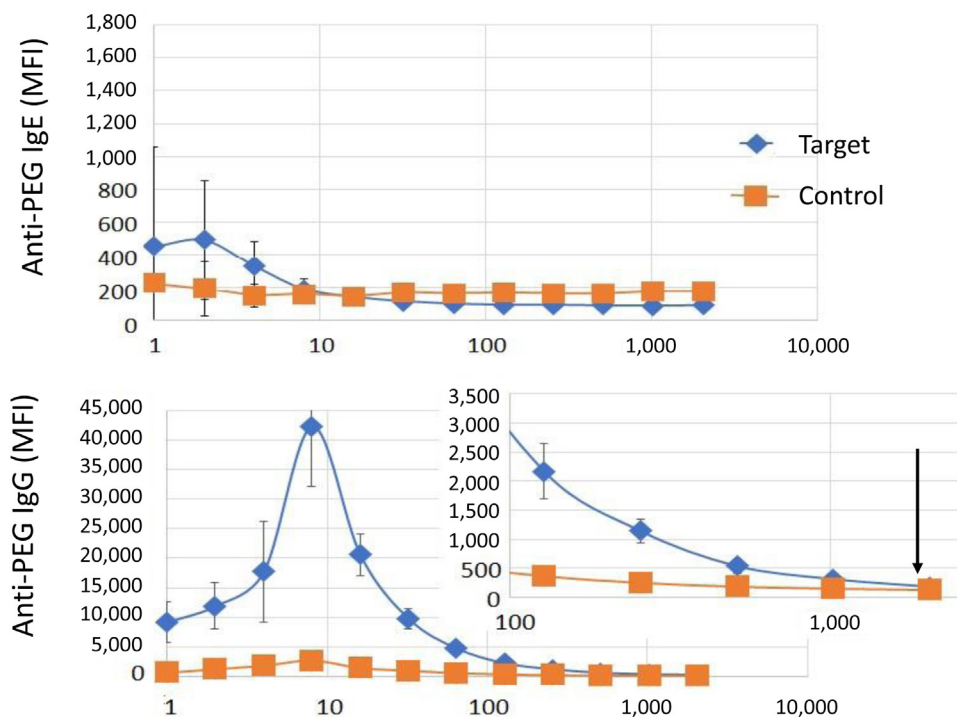
High-affinity murine anti-PEG mAb-conjugated functional CBA beads were prepared using sulfo-SMCC chemistry with Functional Bead Conjugation Buffer Set (BD Biosciences) according to manufacturer's instructions. Conjugation was confirmed by flow cytometry using goat antimouse IgG-PE and the signal to background fluorescence increased by more than 10,000. CBA functional beads E4 and E8 were conjugated with the anti-PEG mAb following the same procedure. E4 beads were incubated with peginesatide (40KD PEG, used for normal serum screening) or pegloticase (multiple 10KD PEG

chains, used for clinical case detection) in a PBS buffer containing 2 mM EDTA and 1% BSA for 1 hour at room temperature with shaking, followed by washing to remove unbound PEGs. E4 beads with a captured PEG product were defined as target beads. E8 beads conjugated with the same anti-PEG antibody but without a captured PEG product were used as control beads.

Screening for anti-PEG antibodies in serum or plasma samples was done using 5 mL Falcon flow tubes or 96-well U-shaped plates for high-throughput runs. For every 96-well plate, approximately  $10^6$  target and  $10^6$  control beads were added to 2 mL serum enhancement buffer (supplied in the BD human CBA kits). Target beads and control beads were mixed in a total of 5 mL buffer (2 mL serum treatment buffer, 2 mL beads capture buffer, 1 mL sample diluent). Then, 50  $\mu$ L of beads and 100  $\mu$ L of serum sample were added per well (96-U plate), incubated for 3 hours at room temperature (or overnight at 4°C) with shaking, and washed twice. Two  $\mu$ L/well of antihuman IgE-PE, antihuman IgGfc-PE, or antihuman-IgM-V450 was added and after 1 hour, the wells were washed twice and analyzed by flow cytometry. Anti-PEG-bio clone B141M (LifeSpan BioSciences, Inc) was used as a positive control. Samples were serially diluted to determine antibody titers, and specificity was confirmed by competition with free PEG.

Anti-PEG antibody specificity in positive samples was confirmed by free antigen inhibition assays. The anti-PEG signals of the target beads were inhibited by PEGylated products (self or other PEGylated products, eg, pegloticase and pegfilgrastim-inhibited signal of peginesatide-target beads), pure PEG (eg, 1KD, 8KD), but not by protein portions of PEGylated products.

### Control PEG3



### Serum titration factor

**FIGURE E1.** Control sample PEG3 is positive only for anti-PEG IgG. The arrows in insets indicate the detection limits. *MFI*, Median fluorescence intensity.

**TABLE E1.** Clinical cases and controls of PEG-associated anaphylaxis

Sample IDs	Potential culprit drugs	Clinical	Skin test material	Skin test results	Reference
PEG1 PEG2 PEG6	Colonoscopy preparations (active ingredient PEG)	Anaphylaxis	Steroid-containing PEG 3550	1 positive 1 negative 1 not tested	Stone et al <sup>E1</sup> communication
PEG7	Steroid injections (excipient PEG)		Steroid-containing polysorbate 80 Steroid-containing neither PEG nor polysorbate 80	2 positives 1 not tested 2 positives 1 not tested	
PEG3 PEG4 PEG5	Colonoscopy preparations (active ingredient PEG)	Controls	Polyethylene glycol/ polysorbates	All negative	Stone et al <sup>E1</sup> communication
PEG8 PEG9	PEGylated liposomal echocardiogram contrast PEG-containing lubricating gel used for a transvaginal ultrasound	Anaphylaxis  Anaphylaxis	PEG  Gel samples and PEG	Positive  Positive	Krantz et al <sup>E2</sup> Jakubovic et al <sup>E3</sup>

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